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Cell culture of THP-1 monocytes and differentiation into macrophages with PMA

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We use this protocol and it's working

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Abstract

THP-1 is a human monocytic cell line originally isolated from a 1-year old male subject with acute monocytic leukemia (Tsuchiya et al., 1980). The cells are easy to culture and are safe, as no viruses have been reported to be present in the cells (Chanput et al., 2014). THP-1 monocytes are round, floating cells with a doubling time of 35-50 h (Chanput et al., 2014). They have been used as a model for studying immune response, monocyte and macrophage function, lipid metabolism and functions of drugs (Chanput et al., 2014). THP-1 cells can be differentiated into macrophages for use in experiments. As macrophages, the cells become adherent to the cell culture vessel. Agents used for differentiation of THP-1 cells include phorbol 12-myristate 13-acetate (PMA), vitamin D, and macrophage colony-stimulation factor M-CSF (Chanput et al., 2014). This cell line is sensitive to culture conditions; e.g. the cells react differently to differentiation substances depending on the cell density (Liu et al., 2023). The protocol presented herein is routinely used for culturing and differentiating THP-1 cells with PMA.

Materials

Equipment:

- Biological safety cabinet
- Cell culture CO₂ incubator (37 °C and 5 % (v/v) CO₂ atmosphere)
- Water bath
- Centrifuge
- Inverted microscope
- Neubauer counting chamber and cover slips or cell counter
- Freeze container (e.g. Mr. Frosty, Nalgene)
- - 80 °C freezer
- Liquid nitrogen tank
- Personal protective equipment (sterile gloves, laboratory coat, safety visor, etc.)

Materials:

- Cell culture plates or flasks (6-well plate: TPP92406, 24-well plate: TPP92424; 96-well plate: TPP92696; 25cm² flask: TPP90026; 75cm² flask: TPP90076; 150 cm² flask: TPP90151)
- Sterile serological pipette
- Sterile Pasteur pipette
- Sterile filter tips
- Sterile falcon tubes (15 and 50 ml)
- Sterile cryotubes (2 ml)

Reagents:

- RPMI-1640 medium (Sigma-Aldrich; cat. no. R8758-500ML)
- FBS Superior (Merck; cat. no. SO615;)
- Penicillin-streptomycin-glutamine mix (Merck; cat. no. G1146)
- PMA (Sigma-Aldrich; cat. no. P1585-1MG)
- Sterile dimethyl sulfoxide (Carl Roth)
- 2-Propanol (Carl Roth)

Cells

- THP-1 monocytes (CLS; cat. no.: 300356)

Safety warnings

- ❗ PMA is toxic when inhaled, swallowed and when it comes in contact with the skin. It is suspected to be carcinogenic. Safety gloves, protective clothing and eye protection glasses should be worn when handling PMA. β-mercaptoethanol is also toxic and its target organs are the respiratory system, central nervous system, skin, gastrointestinal tract and the eyes. It is also combustible. Personal protective equipment should be used when handling β-mercaptoethanol and work with β-mercaptoethanol should be carried out under a laboratory hood.

Before start

Note: All steps with cells should be carried out using a safety cabinet to avoid contamination of cells.

Thawing of THP-1 cells

- 1 Transfer the cryotube containing THP-1 monocytes from the liquid nitrogen tank to the cell culture lab on ice.

Note: Wear protective gloves, goggles and a lab coat when handling liquid nitrogen to prevent burns.
- 2 Thaw the cells immediately in a 37 °C water bath until only a little chunk of ice is visible.

Note: This procedure and the next steps must be performed quickly because the cryoprotectant dimethyl sulfoxide (DMSO) is cytotoxic above 4 °C.
- 3 Prepare RPMI-1640 cell culture medium supplemented with FBS and PSG with final concentrations of 10 % (v/v) and 1 % (v/v), respectively.
- 4 Mix the thawed cells (1 ml) with 9 ml of pre-warmed supplemented RPMI-1640 medium in a 15 ml tube and resuspend thoroughly.
- 5 Centrifuge at 300 x g for 5 minutes at room temperature and carefully aspirate the supernatant with a sterile Pasteur pipette.
- 6 Resuspend the cell pellet in 5 ml of supplemented RPMI-1640 medium and transfer the cell suspension to a 25 cm² cell culture flask.
- 7 Place the flask in a 37 °C incubator with 5 % CO₂ atmosphere.
- 8 Leave the cells in the incubator and observe their growth over a period of days. When the cells have grown and are about 80 % dense, transfer them to a bigger flask and add fresh supplemented RPMI-1640 medium; e.g. cells can be transferred to a 75 cm² cell culture flask with 25 ml of fresh supplemented 1640 medium added .
- 9 The cells should be split twice a week, at a density of around 80 %.

Passaging of THP-1 cells

- 10 Prepare the supplemented RPMI-1640 medium by mixing RPMI-1640 with FBS and PSG to final concentrations of 10 % (v/v) and 1 % (v/v), respectively.



- 11 Evaluate cells using an inverted microscope. Cells should be round, bright and floating; there should be no bacterial or fungal contamination or irregular-shaped floating debris (see **Figure 1.A**). The cells should typically not clump together.
- 12 Collect cells in a 50 ml falcon tube.
- 13 Centrifuge at 300 x g for 5 minutes at room temperature.
- 14 Aspirate the medium and resuspend the cells with 5 ml of fresh supplemented RPMI-1640 medium.
- 15 Prepare a dilution of the cells for counting (1:30 to 1:50 dilution is recommended).
- 16 Count the cells under the microscope using a Neubauer counting chamber or a cell counter; typically, approximately 55×10^6 cells $\pm$ 20 % should be in 50 ml of medium.
- 17 Prepare a new flask of cells by splitting the cells 1:5 in 50 ml of fresh supplemented RPMI-1640 medium.

Differentiation of THP-1 monocytes to macrophages

- 18 Prepare PMA stock solution: PMA is dissolved in DMSO to a final concentration of 1 mg/ml.

Note: PMA should not be exposed to light. It should be aliquoted and stored at -20° C; it should not be subjected to several freeze-thaw cycles to preserve its efficacy.
- 19 Prepare β -mercaptoethanol stock solution: β -mercaptoethanol is diluted with water to a final concentration of 2.5 M, and stock solution can be stored at 4° C.

Note: β -mercaptoethanol should be handled under the biological safety cabinet because its vapors are toxic.
- 20 Collect cells in a 50 ml falcon tube.
- 21 Centrifuge at 300 x g for 5 minutes at room temperature.

- 22 Aspirate the medium and resuspend the cells with 5 ml of fresh supplemented RPMI-1640 medium.
- 23 Count the cells with a microscope using a Neubauer counting chamber or a cell counter.
- 24 Seed cells by mixing the required number of cells for seeding with the required volume of differentiation medium in a 50 ml falcon tube.
- 25 Dispense the appropriate volume of the cell-medium mixture into appropriate vessels.
- 26 The following steps are for seeding two 25 cm² flasks with 3.3×10^6 cells each as an example. Since two flasks require 6×10^6 cells, prepare the cell suspension with an additional 10 % , i.e 7.26×10^6 cells.

27 Calculations

Required volume of cell suspension (number of cells needed ÷ number of cells counted):

For example, if the number of cells counted is 12×10^6 cells, the volume of the cell suspension needed is: $7.26 \times 10^6 \div 12 \times 10^6 = 0.605$ ml of cells.

Required volume of RPMI-1640 medium: 5 ml of medium is used to seed 3.3×10^6 cells in a 25 cm² flask. For this, 5 ml x 2 flasks (+ 10 % extra) = 11 ml of medium. Next, subtract the volume of cells needed to get the final volume of medium needed, i.e 11 ml of medium-0.605 ml of cells = 10.4 ml of medium.

Required volume of PMA stock solution: A final concentration of 100 ng/ml PMA is used for differentiation. In the example, 1.1 µl of the 1 mg/ml PMA stock solution is needed.

Required volume of β-mercaptoethanol stock solution: A final concentration of 50 µM of β-mercaptoethanol is used for differentiation. In this example, 0.22 µl of the 2.5 M β-mercaptoethanol stock solution are needed.

- 28 Mix the required volume of the cell suspension with supplemented RPMI-1640 medium containing 100 ng/ml PMA and 50 mM β-mercaptoethanol In a labeled falcon tube.
- 29 Resuspend properly and dispense 5 ml into each 25 cm² flask for subsequent experiments.

- 30 Gently swirl flasks and place flasks in an incubator at 37 °C with 5 % (v/v) CO₂ atmosphere for 96 h.
- 31 After 96 h, THP-1 cells are fully matured into macrophages and firmly attached to the bottom of the culture vessel (**see Figure 1.B**). Cells can be now used for subsequent experiments.
- 32 **Table 1** provides an overview of the recommended number of cells to seed in vessels of different sizes and the required volumes of supplemented RPMI-1640 medium.

Note: The cell density can be adjusted to suit the requirements of an experiment.

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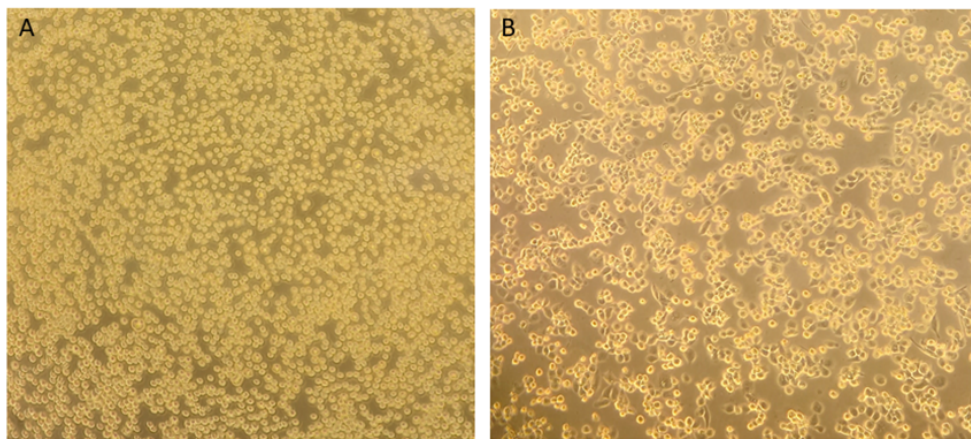


Figure 1: Morphology of THP-1 cells under inverted microscope (magnification: 10 x). A: Monocytes B: Macrophages after 96 h of differentiation with PMA.

- 34 **Table 1: Recommended number of cells to seed in vessels.**

	A	B	C
	<i>Multiwell plates</i>	<i>Cell number [*10⁶]</i>	<i>Volume of RPMI-1640 medium [ml]</i>
	6 well	1.4	3
	12 well	1.0	2
	24 well	0.38	2
	96 well	0.057	0.350
	<i>Flasks</i>	<i>Cell number [*10⁶]</i>	<i>Volume of RPMI-1640 medium [ml]</i>



	A	B	C
	25 cm ²	3.3	5
	75 cm ²	10	15
	150 cm ²	20	30

Freezing of THP-1 cells

- 35 Prepare freeze container (e.g. Mr. Frosty, Nalgene) with 2-propanol and cool it to 4 °C.

Note: Cooling in a freeze container with 2-propanol ensures a slow and gentle temperature decline. A cooling rate of -1 °C/min is the optimal rate for cell preservation.
- 36 Prepare freezing medium: RPMI-1640 containing 20 % (v/v) FBS, 0.1 mg/ml PSG and 10 % (v/v) sterile DMSO.
- 37 Collect cells in a 50 ml falcon tube.
- 38 Centrifuge at 300 x g for 5 minutes at room temperature.
- 39 Aspirate the medium and resuspend the cells with 5 ml of fresh supplemented RPMI-1640 medium.
- 40 Count the cells and calculate the number of needed cryotubes and volume of freezing medium. Label cryotubes.
- 41 Adjust cell concentration to 10×10^6 cells/ml and dispense 1 ml per cryotube.
- 42 Place labeled cryotubes in freeze container and store container at -80 °C overnight before transferring the cryotubes to a liquid nitrogen tank.



Protocol references

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